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Research Article

**PHYTOCHEMICAL STUDY, VITAMINS CONTENT AND FREE
RADICAL SCAVENGING ACTIVITY OF *BIXA ORELLANA* L.
LEAVES FROM BENIN****F. T. D. BOTHON^{1*}, J. ADOVELANDE², C. P. AGBANGNAN DOSSA¹, F. AVLESSI¹, D.
V. WOTTO³, D. C. K. SOHOUNHLOUE¹**¹Laboratoire d'Etude et de Recherche en Chimie appliquée, Ecole Polytechnique d'Abomey Calavi, Université d'Abomey-Calavi, 01 BP 2009 Cotonou, Bénin²Laboratoire de Biochimie et de Biologie cellulaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Bénin³Laboratoire de Chimie Physique, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Bénin.**Abstract:**

This paper reports metabolites content, minerals, vitamins content and radical-scavenging potential of Bixa orellana L. leaves. The phytochemical screening based on the protocols available in the literature, showed the presence of bioactive metabolites such as: polyphenols, alkaloids, saponins, tannins, steroids, anthraquinones, sterols, terpenes and reducing sugars. Flame atomic absorption spectroscopy analysis revealed that the leaves are rich in: calcium (1.56%), potassium (1.54%), magnesium (0.501%) and phosphorus (0.37%). Kjeldahl method showed the richness of the leaves in total protein content (23.70 %). In contrast to their protein content, vitamins A and C content of Bixa orellana leaves is very low: 0.23 µg/g of vitamin A and 6.80 µg/g of vitamin C. Its ethanolic extract has the same IC₅₀ as ascorbic acid for free radical scavenging activity by DPPH method. From these results, it appears that Bixa orellana leaves, in defiance of their medicinal use, can also be used in human nutrition as a source of protein and some minerals.

Keywords: *Bixa orellana, DPPH, metabolites, minerals, vitamins.****Corresponding author:****F. T. D. BOTHON,**

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INTRODUCTION:

Bixa orellana (Bixaceae) is a plant native to Brazil but grows in other regions such as South America (Ecuador, Mexico, Peru), Asia (India, Indonesia) and Africa (Benin, Ghana, Kenya and Nigeria) [1]. This plant is known for its antimycobacterial, antibacterial, antioxidant, antihistamine, neuropharmacological, anticonvulsant, analgesic antidiabetic and anticarcinogenic effects [2-9]. It is a domesticated plant which is found everywhere in Benin [10]. Among the different parts of the plant, seeds are especially the most used; its pigment serves to color the fresh cheese and sauce; and is also used in cosmetics [11]. In Collines region, central of Benin 15 percent of households use *Bixa orellana* (Annatto) fruit as condiment, replacing tomatoes in sauce [12]. The seeds of this plant are a source of natural dye, rich on the carotenoids bixin (dark red color) and norbixin or orelline (yellow color) which are mainly used to develop attractive colors in dairy products (cheeses, margarine, and butter) [13]. The importance of the seeds makes that leaves are under exploited in Benin whereas they are used in by a fringe of the population against vomiting, expectorant, diabetes, ulcer [14]. The aim of this work was to explore other possibilities of using annatto leaves, through the determination of their phytochemical composition and their antioxidant activity.

MATERIALS AND METHODS:

Plant material

Bixa orellana leaves were collected from Valley farm located in the village of Lobogo, Bopa, department of Mono in South Western of Benin in September 2017.

Preparation of plant extracts

The collected leaf samples were dried at room temperature for 7-10 days and crushed to powder form. Hydro-ethanolic extract were obtained after soaked 10g of powdered leaves sample with 100ml of hydro-ethanolic (50/50) solution and stirred for 24 hours. The extract was filtered, dried and stored until further used.

Phytochemical screening

hydro-ethanolic extract of the leaves were submitted to qualitative phytochemical analysis using the methods variously described by Bothon (2014) [15] and Aswathi (2017) [16], to detect bioactive metabolites. The quantitative analysis of phenolic compounds was carried out by spectrophotometry.

Test for alkaloids

Alkaloids were revealed by Mayer's test: A fraction of extract was treated with Mayer's test reagent (2.7 g

of mercuric chloride and 10 g of potassium iodide in 20 ml of water) and observed for the formation of white or yellow-white precipitate in the presence of alkaloid.

Test for flavonoids

add a few drops of a 1/10 soda solution to a few ml of the extract solution. The yellow-orange color characterizes the presence of flavonoids.

Test for anthocyanins

2 mL of aqueous extract is added to 2 mL of 2N HCl. The appearance of pink-red turn's Blue-violet by the addition of ammonia indicates the presence of anthocyanins.

Test for leucoanthocyanins

5 mL of extract added to 5 mL of isoamyl alcohol. Upper layer appears red color indicates for presence of leucoanthocyanins.

Test for anthraquinones

To the chloroformic extract, 10% (v/v) of aqueous KOH is added. After stirring, the presence of anthraquinones is confirmed by a shift from the aqueous phase to the red.

Test for reducing sugars

To 5 ml of the decoction add 1 ml of Fehling reagent (copper sulphate and double tartate of sodium and potassium v/v and heat it all in the bath for 15 minutes.). Obtaining a red brick precipitate indicates the presence of reducing compounds.

Test for tannins

Aqueous extract (5 ml) was mixed with 1ml of dilute aqueous 1% of ferric chloride solution. A dark green or blue green coloration was regarded as positive for the presence of tannins.

Test for saponins

Were determined by Frothing test / Foam test: 0.5mL of filtrate with 5mL of distilled water and shake well. (Persistence of frothing indicates the presence of saponins).

Test for coumarins

The detection of coumarins was carried out by extracting 0.5 g of sample in 10 mL of ether. The suspension was filtrated, evaporated and 2 mL of distilled water were added. The observation of fluorescence was performed by UV light at 365 nm.

Test for terpenoids

Liebermann-Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of

H₂SO₄ was added and observed for the formation of dark green colour.

Test for cyanogenic derivatives

To 1 g of powder, add 5 ml of a mixture of equal volume of water and toluene. Shake well and clean the upper part of the tube, a microporous paper is fixed with a cap at the top of the tube (without soaking in the solution). The red color more or less fast paper indicates the presence of cyanogenic glycosides.

Test for mucilage

Were determined by observing the viscosity after addition of absolute ethanol.

Total phenolic content

Total phenolic contents were determined using Folin-Denis' reagent method [17]. Gallic acid was used as a reference and for the calibration curve; results were expressed as mg Gallic Acid Equivalents per g of Dry Weight (mg GAE / DW).

Tannins content

Tannins content was determined according to Agbangnan (2013) [18]. The tannins content was calculated from Catechin standard curves and expressed as mg Catechin equivalents per g of Dry Weight (mg CE/g DW).

Flavonoid content

Total flavonoid content: The total flavonoid content was determined according to Enujiugha (2012) [19] using AlCl₃. The flavonoid content was calculated from a quercetin standard curve and expressed as mg Quercetin Equivalents (mg QE/g DW)

Physicochemical analysis

Dry matter and ash content

Using the powdered leaves, the dry matter content was determined according to the method AOAC and Ash content was obtained after calcination in the oven at 290 to 600°C following according to AOAC method [20].

Vitamin A and C content

Vitamin C contained in analyzed samples was determined by titration with thiosulfate and diiodine in the presence of starch [21].

Proteins content

The crude protein content was determined from nitrogen content, which was determined using Kjeldahl method [22].

Minerals content

Bixa orellana leaves were reduced to ashes after cremation; ashes were digested for 30 min in a mixture of nitric acid 1M and hydrochloric acid 3N. The filtrates were used to determine minerals content, according to the standards: NF EN 14082 [23] and ISO 15587-2 [24] methods, using flame atomic absorption spectroscopy (VARIANT with spectra A110 software).

Free radical scavenging assay

Free radical scavenging activity of the hydro-alcoholic extract was evaluating using 2, 2-diphenyl-1-picrylhydrazil (DPPH), as described by Bothon *et al.* [15] with slight modifications. 200µl of the different concentrations (1-100 µg/ml) of the extract was added to 2.8 mL of DPPH solution at 120 µM. Ascorbic acid was used as positive control. Absorbance at 517 nm was determined after 1 hour, and IC₅₀ (Inhibitory concentration 50%) was determined. IC₅₀ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

RESULTS AND DISCUSSION:

The phytochemical screening of *Bixa orellana* hydro-alcoholic extract revealed presence of polyphenols (tannins, flavonoids), alkaloid, anthraquinones, reducing sugars, saponins, sterols and terpenoids; whereas anthocyanins, leuco-anthocyanins, mucilage, coumarins and cyanogenic derivatives were absent (Table 1). This result is consistent with that of Gupta (2016) [25]. The quantification of total polyphenol, flavonoids, and tannins contents allowed having the results recorded in table 2. Consumption of polyphenols has been linked to lowered risks of many chronic diseases including cancer, cardiovascular diseases, chronic inflammation and many degeneration diseases [26]. Flavonoids are known for their antioxidant, anticancer and antihyperglycemic property [27, 28]. Apigenin, Luteolin, Ellagic acid were flavonoids identified in the leaves [29]. Terpenes are a big family of compounds a range of pharmacological properties: anticancer, antimicrobial, antifungal, antiviral, antihyperglycemic, analgesic, anti-inflammatory and antiparasitic [30]. Terperne like: Cis-Ocimene, α -Elemene, α -Copaene and α -Carpophyllene was found in the leaves [31, 32]. But no studies have yet been carried out on the chemical structure of the compounds belonging to the families of alkaloids, tannins and saponins in the leaves of *Bixa orellana*.

Table 1: Secondary metabolites of *Bixa orellana* Leaves

Secondary metabolites		
Phenolic compound		+
Alkaloid		+
Flavonoid		+
Anthocyanins		-
Leuco-anthocyanins		-
Anthraquinones		+
Reducing sugars		+
Tannins	Galic	+
	Catechism	+
Sterols and terpenes		+
Mucilage		-
Coumarins		-
Cyanogenic derivatives		-
Saponins		+

(+) indicates present.

Table 2: Polyphenol content of *Bixa orellana* leaves

Polyphenol	Quantity
Total polyphenols	3.081 mg GAE /g DW
Flavonoids	23.35 mg QE/g DW
Tannins	1.84 mg CE/g DW

Minerals, proteins and vitamins are essential nutrients that human body needs in small amounts to work properly. The nutriment content of *B. orellana* leaves is presented in Table 3. It can be seen that the leaves flour is a good source of protein, calcium, magnesium, phosphorus and potassium. Calcium is known to be an important key regulator of a variety

of cellular functions [5]. Calcium and phosphorus present in the largest quantity in the structure of the body and in the bones [33]. A diet rich in potassium reduces the risk of hypertension [34], while magnesium is essential for normal neuromuscular function as well as calcium and potassium transport and reduction of blood pressure [35].

Table 3: Nutrients content of *Bixa orellana* leaves

Nutrients	Concentration
Ash content (%)	8.75
Vitamin A (mg/kg)	0.23
Vitamin C (mg/kg)	6.80
Proteins content (%)	23.70
Cadmium (mg/kg)	0.37
Calcium (%)	1.56
Copper (mg/kg)	9.48
Iron (mg/kg)	212.14
Magnesium (%)	0.501
Manganese (mg/kg)	450.57
Phosphorus (%)	0.367
Potassium (%)	1.536
Sodium (mg/kg)	285.53
Zinc (mg/kg)	22.431

The crude protein was 23.7%, a value superior to the protein content of seeds in general (13 to 17%) [36]. It is close to much other vegetable leaf consumed in Benin and in other Africa countries: *Solanum macrocarpum* (4.3%) [37], *Ocimum gratissimum* (1.21- 3.3%) [38, 39] from Nigeria, *Amaranthus cruentus* from Malawi (18.30%) [40]; but similar to Nigeria *Amaranthus cruentus* (23g/100g) [41]. The present study also showed that the leaves richer in calcium (1.557%), potassium (1.54%), magnesium (0.501 %), phosphorus (0.367 %). Those elements play essential roles in maintaining human health. Compared to the work of Dougnon and al., (2012) [42] one *Solanum macrocarpon* leaves from Benin; *B. orellana* leaves are good sources of calcium and potassium. Calcium and phosphorus are the minerals present in the largest quantity in the structure of the

body and in the bones [43]. Potassium is crucial to heart and smooth muscle contraction [44], a diet rich in potassium reduces the risk of hypertension. Increased dietary magnesium intake confers protection against the incidence of diabetes, metabolic syndrome, hypertension, and cardiovascular disease [45]. Minerals values contents obtained in the current study are very low; geographical origin of plants, belonging to the same species can result different concentration of elements depending of soil features and environmental pollution [46]. Cadmium and copper were present in studied leaves but with a lower level than recommended by World Health Organization in raw plant materials: 0.3 mg/kg of cadmium and 20 mg/kg of copper [47].

Regarding its antiradical activity, the hydroalcoholic extract of *B. orellana* has the same Inhibitory Concentration (IC_{50}) as the standard ascorbic acid (5 μ g/ml) (Figures 1 and 2).

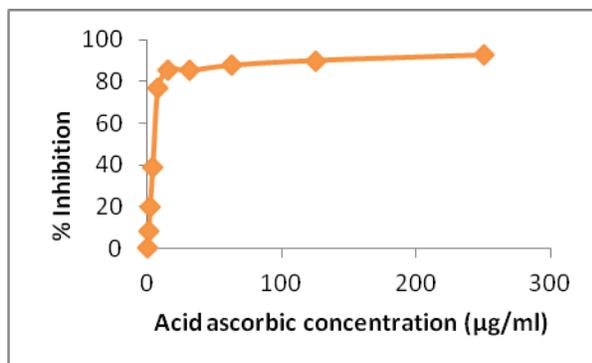


Figure 1: DPPH scavenging by Acid ascorbic

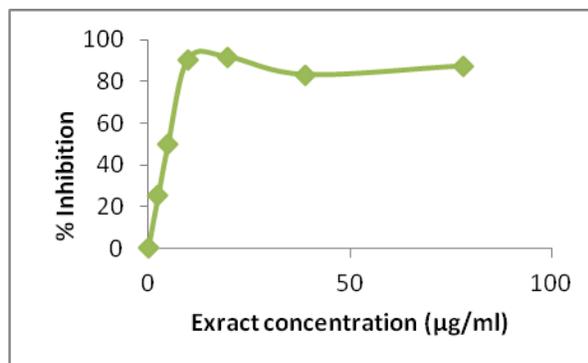


Figure 2: DPPH radical scavenging by *B. orellana* leaf extract

Prevention of the initiation step of oxydation chain by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action [48]. Several studies have been interested in the radical scavenging capacity of *B. orellana* seed [48-50] and find it highest activity. But few studies examined the same activity for the leaves: Shilpi [51] work revealed that methanol extract of the leaves had radical scavenging properties in the DPPH assay with $IC_{50} = 22.36 \mu$ g/ml; when ascorbic acid, used as the positive control had an IC_{50} value of 4.33 μ g/ml. Enciso [52] showed that the hydro-alcoholic extract from leaves of *B. orellana* contain a high concentration of flavonoids which reflected the high correlation with the antioxidant capacity tested. This

correlate with present work result with flavonoids content of 23.35 mg QE/g DW and ascorbic acid content of 6.80 mg/kg. The DPPH scavenging data suggest that hydroalcoholic extract from leaves of *B. orellana* is capable to preventing the initiation and propagation of free-radical-mediated chain reactions [50].

For this fact, the hydroethanolic extract of *B. orellana* leaves can play important role in the treatment of several diseases caused by oxidative stress.

CONCLUSION:

This study suggested that *Bixa orellana* leaves from Lobogo (Benin) are good sources of proteins,

calcium, magnesium phosphorus, potassium and vitamin C. It could be a potential source of natural antioxidant that could replace the use of toxic synthetic antioxidants. They should therefore be planted more in developing countries for not only their already known medical properties but also for their use as vegetables.

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